

The effect of irrelevant visual experience on visual memory

By

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Abstract

Consolidation of memories for long term storage involves increases in excitatory synaptic strength and connectivity between neurons encoding a novel experience. The increase in neuronal excitability caused by memory consolidation could augment excitability induced by the experience of related stimuli irrelevant to the memory. Therefore, the additional neuronal excitability caused by memory consolidation could perturb neuronal activity homeostasis towards higher neuronal activation levels. Under conditions of neuronal hyperactivity, such as in Alzheimer's disease, an increase in excitation induced by memory consolidation would further destabilize homeostasis. We hypothesize that memory deficiency, which would result in reduced neuronal excitability, is an adaptation to maintain neuronal activity homeostasis. To test this hypothesis and to identify whether experience-evoked activity contributes to memory impairments, we used a visual recognition memory (VRM) paradigm that involves synaptic plasticity in the primary visual cortex. In this paradigm, mice are repeatedly presented with a visual grating of a specific orientation and the recognition memory is assessed as a decrease in the exploration of the same stimulus over time. We tested the orientation selective behavioral habituation in a mouse model of Alzheimer's disease (J20 line) and non-transgenic control siblings (wild type). We found that wild type mice display VRM for grating stimulus when tested one day but not at one month after the training period. In contrast, J20 mice did not display VRM even one day after the training period. To examine whether reducing neuronal excitability caused by memory irrelevant visual experience influences the long-term retention of the VRM for grating stimulus, we performed the same task in mice housed in total darkness except during the VRM task. Our preliminary data indicate that dark adaptation rescues the memory deficit in J20 mice whereas disrupts memory in control mice when tested one day after the training. These results suggest that competing experiences promote memory storage in control mice but interferes with it in APP mice.

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1. INTRODUCTION

1.1 Memory acquisition and storage

The ability to learn and remember is critical for survival. Learning is defined as a modification of behavior by experience. The retention of this modification over time is referred to as memory. Memory can be classified based on the retention time, as sensory (retention time: milliseconds), short-term (few seconds), intermediate-term (few hours) and long-term memory (many hours to years) [1, 2]. Such a classification does not address differences in the way the different long-term memories are stored (conscious or explicit memories vs. non-conscious or implicit memories); however, it provides a simple framework for distinct stages of memory.

In the brain, neurons form new connections with other neurons or change the strength of their connectivity in response to changes in experience. That these connections, referred to as synapses, could serve to accommodate new memories was originally proposed by Santiago Ramon y Cajal in 1894 based on his anatomical studies using Golgi staining techniques [3]. In the decades after that, as the workings of the neuron were further elucidated, Donald Hebb put down a postulate that explained how neuronal communication increases the synaptic strength between them leading to learning. To quote Hebb, he wrote “When an axon of cell A is near enough to excite cell B or repeatedly or consistently takes part in firing it, some growth or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.” in his book *Organization of Behavior* [4]. The Hebbian postulate paraphrased as “Cells that fire together, wire together”, remains as the most influential guiding framework for much of neuroscience research on memory storage.

The activity-dependent synaptic strengthening leads to the formation of neuronal assemblies, also called “Engram” cells, during learning. The engrams represent physical correlates of memories, and their reactivation leads to recall of memories. Stabilization of engrams or consolidation of memories involves complex cellular processes that lead to synaptic and network-wide remodeling. Muller and Pilzecker proposed memory consolidation as a theory based on evidence that new information could interfere with the recollection of a recently acquired memory in human subjects [5, 6]. This gave rise to the idea that memories are labile/ fragile upon initial acquisition but strengthened or consolidated over time.

The consolidation of memories occurs at two levels. Synaptic consolidation, which occurs in a time scale of hours, leads to the strengthening of synapses associated with memory storage [7]. Systems consolidation, which occurs over days to years, causes reorganization of memory traces in different brain circuits [8]. Evidence for the ability of synapses to change their strength based on neuronal activity was first obtained by Lomo and Bliss. They discovered that intense electrical stimulation led to the prolonged strengthening of synaptic transmission in the hippocampus [9]. The long-lasting enhancement of activity is referred to as long term potentiation (LTP). LTP requires both presynaptic activity followed by postsynaptic activity and closely follows the Hebbian idea of learning. Pharmacological blockade of protein translation interferes with both LTP and memory storage, suggesting mechanisms associated with late LTP (L-LTP) is associated with the consolidation of short-term memories into long-term memories [10]. Though first discovered in the hippocampus, evidence of LTP has been presented in other parts of the cortex, including the primary visual cortex [11-13]

LTP increases intrinsic excitability, which is the ability of a neuron to respond to external input. Evidence that LTP affects neuronal excitability at the cellular level is shown in layer 5

neurons of the visual cortex [14]. LTP of intrinsic excitability (LTP-IE) leads to a reduction in threshold current required to elicit action potentials, indicating an increased sensitivity for activity. LTP-IE is induced by different forms of learning. Previous visual experience has been shown to increase visual sensitivity in semi-intact tadpoles measured through visual excitation potentials [15]. Pavlovian conditioning in *Hermissenda* increased neuronal excitability by reducing K⁺ conductance [16]. Though first documented in invertebrates, more data suggests that learning causes increased excitability in vertebrates as well [17].

Sensory experience and other forms of learning also result in the formation and maturation of new synapses. In vivo imaging of dendritic spines reveals an increase in the number of new spine stabilization following monocular deprivation, checkerboard whisker trimming, motor learning and fear conditioning [18-22]. In addition to an increase in excitatory synapse connectivity and strength, learning is also facilitated by disinhibition [23, 24]. Overall, these synaptic modifications result in changes to the excitation/inhibition balance (E/I balance) of a neuron. For instance, auditory fear learning is dependent upon the shift of the E/I balance in auditory cortex and hippocampus to facilitate an overall positive effect on associate learning. To avoid epileptic activity, due to increased excitability caused by memory acquisition, neurons shed other excitatory synapses that were present prior to learning. This is supported by the observation that increased spine gain is immediately followed by an increase in spine loss during learning [25]. These synaptic changes are energetically expensive but are important for neuron's homeostasis.

1.2 Forgetting

In the previous section, the impetus was given to how memories are formed and stored by the brain at the cellular and synaptic level. In this section, I reflect on the need for forgetting and important theories regarding forgetting.

At any given point of time, the brain is bombarded with a tremendous amount of information. While considering the brain as a definite information storage device, forgetting is beneficial for memory. Defined by Tulving (1974) as the inability to recall something that could be recalled at a previous moment [26]. Pioneering work in exploring the relationship between learning and forgetting was done by Ebbinghaus in his famous experiment involving non-sensical syllables [27]. He designed an experiment where he trained human subjects on learning non-sense three lettered syllables and tested their memory at different intervals. Corresponding data were plotted as a 'learning curve'. Also called as 'forgetting curve', it showed that following the initial acquisition of memory there is an exponential loss of information in a short period followed by a much slower decay of the memory. Another important conclusion from this experiment was that repetition during learning improves the retention of data over time and is reflected by the less steep slope of the curve. This led to the formulation of the decay theory – that information stored in the brain is gradually lost over time due to the natural turnover of cellular components.

Decay theory has certain criticisms; namely, it determines time as the only factor responsible for forgetting. However, various other intervening factors can cause forgetting for example benzodiazepines are well known to cause anterograde amnesia [28] [29, 30] Another criticism is that it is a passive form of forgetting, where the brain takes no part in controlling what memories are retained vs forgotten. Intuitively, we know that certain older memories are

more robustly retained than others whilst certain recent memories are easily forgotten. This gives support to the idea that, if not all, some forms of memory do not follow the decay theory of forgetting. Decay theory, however, helps explain some aspects of working or short-term memory.

Another prominent theory of forgetting is the theory of interference. Drawing heavily from Müller and Pilzecker's seminal work on memory consolidation, Interference theory can be described as forgetting due to incomplete memory consolidation [31]. In the previous paragraphs, I describe the idea that during memory consolidation, an otherwise fragile memory is stabilized over time. In its fragile state, memory is affected by a variety of factors, one of which is new incoming information. This is called Proactive Interference [32]. If the interference is caused by an already existing memory, the interference is called Retroactive interference [33, 34]. There is evidence that induction of LTP can itself act as an interference leading to partial forgetting.

When rats underwent novel exploration that gave rise to entirely new LTP in the hippocampus, their memory for hidden platforms decreased in Morris water maze task [35].

In contrast to natural decay and interference mediated memory loss, forgetting could also be intentional. Motivated forgetting occurs when memories are suppressed through cognitive control during acquisition or consolidation [36, 37]. In addition to memory loss due to loss of engrams, forgetting can also occur due to a failure of retrieval of information from stable engrams [38].

1.3 Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia, affecting 10% of the elderly > 65 years [39]. By 2050, the number of affected is expected to rise to 14 million in the US. AD is

a slowly progressing form of dementia and cognitive decline with three phases, an early phase, a middle stage (Mild Cognitive Impairment, MCI) and a late stage, that are not clearly discernable. Recent work shows that subtle memory deficits start 2-3 decades before more severe symptomatology emerge [40]. As a result, diagnosis of AD happens well past the MCI stage.

While the search for reliable biomarkers goes on, the focus has been shifted towards the pre-symptomatic stages of AD as a target for intervention.

More than ninety percent of AD is of late-onset with unknown etiology. [41]. A small fraction of AD is caused by dominantly inherited mutations (Familial AD or FAD). Regardless of the kind of AD, two central pathological hallmarks are the presence of A β plaques and Tau neurofibrillary tangles. In 1984, George Glenner & Caine Wong were able to first isolate a sticky beta pleated sheet from meningeal blood vessels of AD patients [42], later identified to be a molecule called A β [43]. The A β peptide is a byproduct of proteolytic cleavage of a larger protein called amyloid processing protein (APP) by an enzyme gamma secretase [44]. Interestingly, many mutations associated with FAD are identified in genes coding for APP and presenilin (PSEN), a core component of gamma secretase [45]. These mutations increase the production of longer A β peptides, which have higher propensity to form A β plaque [46]. Further evidence for a causal role for APP in AD comes from observations in patients with Down syndrome (Trisomy 21;[47]). The gene coding for APP lies on chromosome 21 and in patients with Down's syndrome, APP production is upregulated. Consequently, patients with Down's syndrome develop neuropathological features akin to AD. This evidence led to the formulation of amyloid cascade hypothesis for the progression of AD, where A β deposition plays a central role in guiding the pathology [48-55].

Variants of A β are endogenously produced and are believed to play a role in normal

synaptic function. The effect of A β , however, is dose-dependent. At picomolar concentrations, A β increases LTP and improves learning and memory. In contrast, at nanomolar concentrations, it impairs LTP and cognition. [56]. Higher levels of A β reduces synaptic transmission and induce excitatory synapse loss [57]. Excitatory synapse loss correlates more strongly with the cognitive decline in AD patients than amyloid plaques [58].

Human AD-associated mutations in APP and PSEN have been introduced in mice. These mice (APP mice) serve as a model to investigate the mechanisms of synaptic dysfunction.

However, most studies of synaptic dysfunction are limited to ex-vivo preparations from these mice. They reveal synaptic plasticity deficits, such as impairments in long-term potentiation, facilitation of long-term depression, and a reduction in synapse density [59-64]. Advancements in optical imaging techniques have allowed visualization of fluorescently labeled neurons in vivo in APP mice [65]. These studies confirm that dendritic spines, which are postsynaptic sites for excitatory synapses, are lost at a higher rate in APP mice than wild type controls.

1.4 Neuronal hyperactivity in Alzheimer's disease

For a while, the consensus was that AD led to decreased neuronal activity [66, 67]. Advances in human brain imaging techniques in the last decade have helped identify an initial hyperactive phase in AD. In humans, studies on asymptomatic offspring (age>50 years) of late-onset sporadic AD patients (the disease confirmed by autopsy) show an extensive activation of frontal and temporal lobes especially hippocampal areas during paired-associates memory tasks compared to controls [68]. This increased activation coincided with decreased activity in cingulus and thalamus during encoding and recall [68]. This indicated an underlying pattern of perturbed activity decades before

the actual disease presents itself overtly in patients with a familial risk of AD. Similarly, people with genetic mutations that predispose them to AD show aberrant activation of cortical networks [69], suggesting an imbalance in excitation and inhibition (E/I). Consistently, humans and mice with APP mutations exhibit a high incidence of seizures [70].

Neuronal hyperactivity directly impairs memory in MCI patients with hippocampal hyperactivity where deficits in pattern separation have been demonstrated [71]. Overactivation of hippocampal circuits and memory impairment were reversed with Levetiracetam, an anti-epileptic drug. These studies further strengthen the idea that hyperactivity affects cognition [72]. The connection between hyperactivity and deficits in memory is also seen in other diseases like epilepsy. AD patients who exhibit epileptic activity display memory deficits earlier than the ones that do not, indicating epileptic activity aggravates AD in these patients [73].

Like AD patients, APP mice exhibit hyperactivity preceding plaque formation and cognitive decline [74]. In a double transgenic (APP and PSEN) mouse model, soluble A β directly causes hyperactivation of hippocampal neurons [69]. Interestingly, suppression of APP expression after plaque formation reduces neuronal hyperactivity, suggesting that APP rather than A β plaque play a causal role in evoking hyperactivity. Dendritic degeneration, increased glutamate release, and a reduction in inhibition of excitatory neurons have been proposed to cause hyperactivity in APP mice [75].

On a molecular level, Δ FosB could explain how hyperactivity could directly affect memory acquisition. Δ FosB is a transcription factor produced in response to stimuli like stress, and drugs [76]. Δ FosB overexpression in mouse hippocampus impairs memory and increases the number of immature dendritic spines [77]. Interestingly, Δ FosB has an unusually long half-life (several weeks) and consequently, it accumulates over time causing persistent effects in hippocampal

activity [76]. Induced seizures in wild type mice show an increase in Δ FosB production [76]. In multiple mouse models of AD, higher frequency EEG recordings from dentate gyrus correlated with higher levels of Δ FosB and memory impairments. These results indicate that hyperactivation in AD could affect memory at the transcriptional level through Δ FosB.

1.5 Mouse models for AD – J20 and 5X FAD

J20 mice overexpress APP with KM670/671NL (Swedish), APP V717F (Indiana) mutations found in humans with FAD [78]. This model is well characterized for its synaptic [79] and cognitive dysfunction with progressive AD pathology [78]. J20 mice exhibit learning deficits[80], excitatory synapse loss [81], neuronal hyperactivity, altered E/I balance and increased Δ FosB expression in the dentate gyrus[76].

5X FAD mice overexpress mutant forms of human APP with K670N, M671L (Swedish mutations), I716V (Florida mutation); V717I (London mutation) and mutant PSEN1 (M146L, L286V) [82]. 5X FAD develop AD pathology rapidly with increased intraneuronal levels of A β noticeable at 1.5 months [83]. Plaques are also seen earlier on (2 months) in this model than in J20 and other models of AD which typically take 6 months to develop [83].

1.6 Primary visual cortex as a model system to study memory deficits in Alzheimer's disease

Visual dysfunction is prevalent in Alzheimer's disease; however, primary visual cortex is relatively spared earlier in AD [84, 85]. Interestingly, in ~ 5% of patients with amyloid pathology and neurofibrillary tangles, neurodegeneration is most extensive in the primary visual cortex [86]. This variant of AD is referred to as posterior cortical atrophy (PCA) [86]. In the early stages, these

patients exhibit visual deficits but retain normal cognition. It is unclear why amyloidosis affects the visual cortex very early in some patients but not others. Nevertheless, PCA serves to illustrate that visual cortex is vulnerable to amyloidosis. Consistently, mouse models of AD display activity and synaptic deficits in the visual cortex [87-89]. Mouse models of AD display impaired orientation selective tuning of neurons in the primary visual cortex [87]. Also, a fraction of visual cortical neurons that were hyperactive and hypoactive is also higher in these mice [90]. In addition to the relevance of primary visual cortex to amyloid pathology, it is very amenable to study homeostatic plasticity mechanisms because of the relative ease with which visual experience can be manipulated.

1.7 Visual recognition memory in mice

Visual recognition memory (VRM) is thought to be stored in higher order cortices like perirhinal cortex [91]. Recently, synaptic plasticity in the primary visual cortex was found to be necessary for VRM of a grating stimulus [92]. This paradigm exploits the innate tendency of mice to explore a novel environment more than a familiar environment and therefore, could track behavioral habituation to a repeatedly presented grating stimulus of a specific orientation as a readout of memory. High contrast phase reversing gratings of a specific orientation reliably elicit neuronal activation in the primary visual cortex [11, 93]. These visual evoked potentials (VEP) gradually increase with repeated presentation of the same orientation stimulus and saturate over an 8-day training period [94]. This is accompanied by a decrease in the exploration of that particular orientation stimulus [94]. The increase in VEP following repeated exposure to a stimulus is referred to as stimulus specific response potentiation, which uses many of the same machinery necessary for LTP [95]. Interestingly, presenting an orthogonal orientation stimulus to the same

mice produces VEP response comparable to the first exposure of the previous orientation stimulus [94]. Similarly, presentation of orthogonal orientation stimulus following behavior habituation to the original stimulus evokes more exploratory behavior, suggesting that the habituation memory is orientation specific [94]. This orientation specific behavioral habituation suggests that primary visual cortical neurons activated by this orientation inhibit curiosity-driven exploration of mice.

1.8 The effect of irrelevant experience on visual memory

Neurons maintain their firing rate within a dynamic range to avoid epileptogenesis. Memory formation transiently shifts the E/I balance of neurons towards more excitation. Since memories are formed within the context of everyday irrelevant experiences, which also increases neuronal excitability, forgetting and memory impairment could be an adaptation to maintain neuronal activity homeostasis. Isolating activity evoked due to memory relevant and irrelevant experiences for most memories are difficult. To overcome this limitation, we used the VRM task that is dependent on synaptic plasticity in the primary visual cortex, which is mostly unimodal (activity is mostly driven by visual experience). This paradigm allows us to deprive visual experience, through dark adaptation, at all times except during the VRM task. Dark adaptation deprives all visual experience including naturally occurring stimuli related (irrelevant experience) to the VRM task. Using this approach, we found that irrelevant experience promote memory under normal conditions but disrupt it under conditions of amyloid pathology.

2. MATERIAL AND METHODS

2.1 Animals

All animal procedures were approved by The Institutional Animal Care and Use Committee at the University of Kansas and meet the NIH guidelines for the use and care of vertebrate animals. Mice are housed in 12 h light/dark cycle unless mentioned otherwise. The J20 [78] and 5X-FAD [82] lines (a kind gift from Dr. ShiDu Yan) are of mixed C57BL6J x DBA2 and C57BL/6 X SJL backgrounds, respectively. J20 and 5X-FAD lines were maintained by breeding heterozygous (Het) males and WT females. WT and Het progenies were used for all experiments. WT female mice used for the crosses were obtained by crossing WT males and females of the same genetic backgrounds.

2.2 Genotyping

Mice genomic DNA extraction. Genomic DNA was isolated from 2mm ear punches following overnight digestion with a lysis buffer (100mM Tris-HCl, pH8.0; 5mM EDTA, pH8.0; 0.2%SDS; 200mMNaCl, 500µl/sample) and Proteinase K (Invitrogen™ Proteinase K, catalog#25-530-049. 10µl/sample) at 56°C in an incubator shaking at constant 225 rpm. On the second day, DNA was precipitated using 500µl of Isopropanol alcohol. The DNA isolated was fished and dissolved in approximately 25 – 100 µl of TE (10 mM Tris-Cl and 1 mM EDTA) overnight in a water bath at 56°C.

Standard PCR reaction for genotyping. PCR reaction was set up using Taq polymerase master mix (Taq DNA Polymerase with Standard Taq Buffer, Catalog#M0273S, New England Biolabs),

with transgene specific and control (connexin) primers (Table 2.2.1), in a thermocycler (SimpliAmp, ThermoFisher Scientific) using strain specific PCR conditions (Table 2.2.2).

Gel Electrophoresis. PCR products were resolved using agarose gel electrophoresis. 2% agarose gels were made with GreenGlo indicator dye (FisherScientific) in 1X Tris Acetate-EDTA buffer (Tris Acetate-EDTA buffer 50X concentrate, Catalog#SRE0033-1L). 1kb ladder was used to determine size of PCR product (Ladder Quick-Load Purple 1 kb DNA Ladder, Catalog#N0552S). The gel was imaged under blue light in Accuris SmartDoc 2.0 System with Blue light illumination base (Accuris Instruments).

Table 2.2.1. Primers used for genotyping are listed below

Sr. No.	Mice strain	Name	Primers sequence
			5' Label Sequence 5' --> 3' 3' Label
1	J20	A4 901-930	GACAAGTATCTCGAGACACCTGGGGATGAG
2		A4 2070-2041	AAAGAACTTGTTAGGTTGGATTTTCGTAGCC
3	5XFAD	oIMR1644	AAT AGA GAA CGG CAG GAG CA
4		oIMR1645	GCC ATG AGG GCA CTA ATC AT
5	WT	Connexin-common	CCA TAA GTC AGG TGT AAA GGA GC
6		Connexin-WT	GAG CAT AAA GAC AGT GAA GAC GG

Table 2.2.2. PCR cycle

Sr. No.	Mice strain	PCR cycle
1	J20	95°C for 30 sec (initial melt) 95°C (30 sec) 56°C (30 sec) 68°C (1 min) x 35 cycles (amplification) 68°C for 7 min (final extension)
2	5XFAD	94°C for 5 min (initial melt) 94°C (30 sec) 58°C (1 min) 68°C (1 min) x 35 cycles (amplification) 68°C for 7 min (final extension)

2.3 Visual recognition memory paradigm

Mouse location tracking. The apparatus used to track mouse location for this paradigm is a force-plate actometer with a square sensing surface measuring 42 cm on each side. Identical monitors, covering the entire side of the apparatus, are placed on two of the sides to deliver the visual stimulation. The other two sides of the arena are made opaque with black paper. Force samples from each of the four force transducers that support the sensing surface will be taken at a rate of 100 samples/s via a USB based data acquisition device controlled by a computer running custom-written Visual Basic software.

A camera (Logitech C920 HD Pro Webcam) was used as a secondary recording instrument as well as to identify the monitor delivering the stimulus. Representative figures for each recording instrument is provided in Figure 1.

Orientation specific habituation. The behavioral paradigm (adapted from , is shown in Figure 2. A. In this paradigm, mice were habituated for two days (30 minutes each) to the chamber with two identical monitors displaying a gray screen on either side. For the next seven days, these mice were exposed to grating stimulus in two 15 minutes sessions, separated by two hours, in the chamber. The stimulus consists of five blocks of 100% contrast sinusoidal grating of a specific orientation that phase reverse at 2 Hz with a 30-second inter-block interval (Figure 2.B). Phase reversing grating stimulus was generated by a custom written MATLAB code (a kind gift from Dr. Mark Bear lab) modified to pseudo randomly display the stimulus in one of the two monitors and to trigger the force plate actometer four minutes before the start of the stimulus. Between the sessions, the chamber was thoroughly cleaned. After initial removal of excrement using a tissue paper, the plate was cleaned with 70% ethanol, Vikron disinfectant and water in that order.

Lastly, a dry paper towel was used to rid the plate from any residual moisture. The room was kept devoid of any external food/animal odor source.

During the sessions, mice were presented with five minutes of gray screen on both monitors followed by five blocks of phase reversing grating stimulus (45°) in one of the randomly chosen monitors. The other monitor remained gray. On the following test day (to assess recent memory) and 30 days later (to test long-term memory), these mice were presented with the same 45° stimulus (two sessions) and a novel 135° stimulus (two sessions).

Analysis of Forceplate Data. The voltage measurements from the four sensors of the force plate is converted into X-Y co-ordinates using the following formula

$$x = (X1 f1 + X2 f2 + X3 f3 + X4 f4) / (f1 + f2 + f3 + f4);$$

$$y = (Y1 f1 + Y2 f2 + Y3 f3 + Y4 f4) / (f1 + f2 + f3 + f4)$$

Where, (f1+f2+f3+f4) = sum of applied forces and f1, f2, f3, f4 are the individual reactive force at each sensor [96]. A moving average of 0.5 seconds was calculated to minimize fluctuations due to electronic noise. The data are further down-sampled from 100 Hz to 2 Hz to minimize movement artifact caused by small body movements (such as during grooming).

The half of the chamber closest to the stimulus monitor is considered the stimulus zone and the other half is the non-stimulus zone. The stimulus zone preference (SZP) index is calculated as the difference in the time mice spent exploring (active exploration at ≥ 3 cm/s) the stimulus and non-stimulus zones divided by the total exploration time. More positive values indicate that the mice spent more time in the stimulus zone.

Novelty Index (NI) is calculated as percentage decrease in time spent exploring (>3cm/s) familiar stimulus zone compared to novel stimulus zone on the same day for each mouse. NI is used as the measure of how mice treated the different stimulus on the same test day. A greater reduction in NI reflects that the mice remembered the familiar stimulus and explored the novel stimulus more on the test day. Smaller reductions reflect the mice did not behave differently in the familiar and novel stimuli.

Mice that did not respond to stimulus on both the sessions on days with a novel stimulus (first day of stimulus, recent memory test day, and remote memory test day when performed) were removed from the analysis. Only data from the first session of first day, recent memory and remote memory test days were analyzed.

Analysis of Video Data. Optimouse – Tracker (MATLAB-based Open source program) [97]

was used to track the mouse body positions on the arena. Mouse body positions were detected using this software from a manually started camera recording for each session. The video files were manually primed using inbuilt functions before applying the detection software. First the spatial and temporal region of interest was defined before manual background subtraction.

Inbuilt functions were used to adjust contrast and brightness to attain optimal detection of mouse body position throughout the video. Files that had completed this priming were then run through the Optimouse detection software. Values in the form of body and nose positions were obtained as raw data in the form of X-Y coordinates. At least 95% of the frames were accurately tracked. Sessions with less than 95% tracking were discarded due to poor tracking and video quality. The values were then converted to the scale of force plate readings using custom excel template for direct comparisons.

2.4 Visual recognition memory with dark adaptation

Home cage movement during 24 h dark cycle. Mice movement in the home cage were tracked

with two passive infrared sensors placed at two different locations on the wire top. Mice movement reliably evoked sensor activity (visual observation) and the time of movement was logged using a precision timer (Adafruit DS1307 Real Time Clock Assembled Breakout Board).

Dark adaptation paradigm. The VRM paradigm with dark adaptation is similar as above except

that mice were housed in 24 h dark room following the first day of habituation to the force plate apparatus (Figure 4). Also, the test day consisted of one familiar (45°) and novel sessions (135°). The mice cages were transported between the behavior room and dark room in an opaque box and covered with a black plastic bag to prevent any light exposure.

Dark adapted mice moved less during stimulus presentation in the VRM task compared to mice housed under 12 h light/dark cycle. Therefore, we only analyzed data from dark adapted mice that moved within the range of movement observed during the stimulus presentation in mice housed under 12 h light/dark cycle experiments. Just as in the 12 h light/dark cycle experiments, mice that responded to stimulus in at least one of the sessions on the first day and recent memory test day were included for the analysis.

2.5 Perfusion

Mice were anesthetized with 2% Avertin solution (Fisher Scientific) and perfused transcardially with PBS and 4% paraformaldehyde (Formaldehyde Solution, min. 37% GR for Analysis, ACS, Reagent Ph Eur Grade, EMD Millipore Catalog#M1040031000,). The brains were harvested and

post fixed for 24 hours in paraformaldehyde solution. The tissue was subsequently sliced in a vibratome at 50µm thickness and the tissues were kept submerged in PBS.

2.6 Immunohistochemistry

Sections were blocked with 10% Normal goat serum in 1% TritonX100/PBS for 2 hours, then incubated with anti-rabbit deltaFosB (1: 1000 dilution, Delta FosB (D3S8R) Rabbit mAb #14695, Cell Signaling Technology) or with anti-mouse clone 6e10 antibody (1:500 dilution, Purified anti-β-Amyloid, 1-16 Antibody Clone 6e10, Biolegend) overnight, and washed with PBS. After further incubation with Alexa Fluor 555 conjugated goat-anti-rabbit secondary antibodies (Thermo Fisher Scientific; 1:400; Cat # A21428; RRID:AB_2535849), and washing, the slices were mounted on slides using Fluoromount-G (Southern Biotech). Images were acquired using an upright fluorescence microscope (Zeiss). Two slices per brain was imaged and analyzed. Immunofluorescence was quantified using imageJ. Briefly, a 0.12 mm² rectangular ROI was placed right below layer 1 and the image within the ROI was background subtracted using the inbuilt function (rolling ball radius of 50 pixels). Mean intensity from two slices per mouse was averaged. The mean fluorescence intensity of WT was used to normalize the fluorescence intensity of each sample (both WT and J20).

2.7 Statistics

Graphpad was used as the statistical software. In each figure, the statistical method used is noted, and a summary of the results from each comparison is listed in List of Tables.

3. RESULTS

3.1 Validation of the coordinates obtained from force plate actometer.

To monitor the location of the mice during the visual stimulus, we used a force plate actometer, which provides a high spatial and temporal resolution map of mice location in the apparatus. We first tested the accuracy of force plate actometer by comparing X-Y coordinates obtained from the force plate measurements with the camera measurements. Due to the positioning of the camera, the coordinates obtained from force plate actometer and the camera are flipped 90° with respect to each other. We found a near perfect correlation ($R = 0.995$, $P \text{ value} = <0.0001$) between camera and force plate X-Y coordinates, suggesting force plate appropriately represent the position of the mice (Figure 4, Table 1).

3.2 Visual recognition memory deficits in normal and amyloid pathology conditions.

One-month old C57BL6J mice have been shown to display orientation specific habituation to a phase reversing grating stimulus. However, it is unclear whether adult mice (3 – 4 months) exhibit orientation specific habituation and whether this memory stays intact as a long-term memory. Also, it is unclear whether this memory is susceptible to amyloid pathology. To test whether adult mice with or without amyloid pathology could form and retain orientation specific habituation, we repeatedly exposed J20 mice and non-transgenic (WT) sibling controls to a phase reversing grating stimulus (45°) for eight days. On the ninth day (recent memory test day), the mice were exposed to the same stimulus and a novel stimulus (135°). One month later, mice were again exposed to 45° and 135° stimulus (remote memory test day).

On the first day of stimulus presentation, both WT (n=9) and J20 (n=12) mice spent more time in the stimulus zone, exploring the 45° stimulus. This is reflected by high SZP values for both groups on day 1. This shows that 3 – 4-month-old mice display curiosity to explore the phase reversing grating stimulus and that APP overexpression does not impair exploratory behavior (Figures 5 and 6). Interestingly, on the test day for recent memory, WT mice display negative preference for stimulus zone, suggesting that these mice avoid the familiar stimulus, an indication of recognition memory (Repeated measures One-way ANOVA, Tukeys post hoc, $p=0.0109$). To test whether this memory is retained for a long period, we presented the same 45° stimulus one month later to the same mice. Interestingly, these mice displayed positive preference for the stimulus exploration, suggesting that the visual recognition memory is impaired one month after the last exposure (Figure 5). In contrast to their non-transgenic siblings, J20 mice with APP overexpression continued to display positive preference to stimulus zone on the recent and remote memory test days, indicating that they did not form a robust visual recognition memory (Figure 6).

To test whether the negative preference for the stimulus zone is purely a reflection of long-term memory or whether there is a working memory component, we compared the SZP for the first two blocks of stimulus (block1+2) with the last two blocks (block4+5) on the recent memory test day for the familiar stimulus (Figure 7). We reasoned that a more positive exploration of stimulus zone in the first two blocks compared to the last two blocks would be indicative of working memory. However, the mice displayed negative preference for the stimulus zone at both times, suggesting that the reduced exploration of the familiar stimulus is not due to working memory (Figure 7). When we compared these groups of blocks (block 1+2 vs block 4+5) for the novel 135° stimulus, we found a comparable positive exploration of the stimulus zone between these blocks. These results suggest that the SZP is not influenced by working memory (Figure 7).

To test whether the reduction in SZP for WT on the recent memory test day is not due to a change in their natural tendency to explore, we presented a novel 135° stimulus on the recent memory test day. Previous work has shown that one-month old C57BL6J preferentially explored the novel angle (135°) of the grating stimulus despite prior exposure to the 45° stimulus. We calculated the Novelty Index (NI), which is the percentage decrease in familiar stimulus exploration compared to novel stimulus exploration on the same day for each mouse. Identical exploration time for the familiar and novel stimuli would yield a NI of zero whereas a more negative NI indicates a reduction exploration of the familiar stimulus relative to that of novel stimulus. On the recent memory test day, WT mice show a greater reduction in NI: – 30% when compared to J20 mice (NI: – 10%) indicating that it was capable of not only remembering the Familiar stimulus (45°) but also was able to distinguish the stimulus from Novel stimulus (135°) (Figure 8). There was no difference in the NI of WT and J20 mice on remote memory test day (Figure 8).

To test whether the change in SZP in WT mice on the recent memory test is due to a change in movement, we measured the average distance travelled through the arena during stimulus presentation on the first day of stimulus (Session 1 - 45°) and the recent memory test (45° and 135°). We did not find any difference in the movement between the first day and test day for WT mice (Figure 9). J20 mice tend to move more but it is not statistically significantly different compared to WT mice (Figure 9).

Response to the stimulus was not dependent upon the side of stimulus presentation.

To ensure that the exploration of novel stimulus is not influenced by the stimulus zone (left vs right side of the chamber), we first quantified whether the stimulus appeared equally on both

monitors (Figure 10 A) Out of a total number of 105 sessions analyzed, the number of sessions with stimulus on the left side of the arena ($n = 54$) was almost equal to stimulus on the right side ($n = 51$). When compared by genotype, out of total sessions ($n = 45$), WT mice had left-side stimulus ($n=22$) and right-side stimulus ($n=23$). J20 mice had similar distribution of stimulus - left-side stimulus ($n=32$) and right-side stimulus ($n=28$).

Next, we tested whether the exploration time is higher on the stimulus side regardless of whether the stimulus appeared on the left or right monitor. Percentage time spent during the stimulus in stimulus side vs non-stimulus side was analyzed separately by genotype. For both genotypes, the mice spent more time in the stimulus side regardless of which side the stimulus came on (Figure.10 B and C). Thus, the effect on SZP was not due to any bias in the recording arena.

3.3 J20-Females show VRM but higher elimination rates due to non-exploratory behavior compared to J20-Males

To test whether VRM is gender specific, we tested J20 and WT females using the same VRM paradigm. Similar aged females WT ($n=4$) and their litter mates J20 ($n=6$) mice were tested. In contrast to male mice (~20% non-exploratory on any of the days with a novel stimulus), five out of the six J20 female mice did not explore both the novel and familiar stimulus, suggesting female mice are not suitable for this task (Figure 11 A). Of the mice that responded to the stimulus, the VRM was similar to that of males (Figure 11 B and C)

3.4 5X FAD-Males show VRM

To test whether the impairment in VRM in J20 mice is a general feature of amyloid pathology and not a strain specific effect, we performed the same task with 5X FAD mice with human FAD mutations in genes coding for APP and PSEN [82]. 5X FAD (n=3) and their WT litter mates (n=2) mice, aged 4 months, were tested for their VRM to the grating stimulus. Our preliminary data is consistent with the results we obtained with J20 mice (Figure 12), suggesting that the long term memory deficit in WT mice and recent memory impairment in J20 mice are not restricted to one genetic background.

3.5 J20 mice show increased Δ Fos-B levels when compared to wild type littermates

APP overexpression has been shown to increase neuronal hyperactivity, which is associated with memory impairments. We first tested whether J20 mice exhibit increased APP expression in the primary visual cortex by immunohistochemistry using an antibody against β -amyloid (brain slices within – 4.55 mm from bregma to – 2.78 mm). We found that, under identical imaging conditions, WT mice did not have any immunofluorescent signal whereas J20 mice had visible fluorescence (Figure 13 A). This qualitative analysis indicated that APP is overexpressed in the visual cortex of J20 mice.

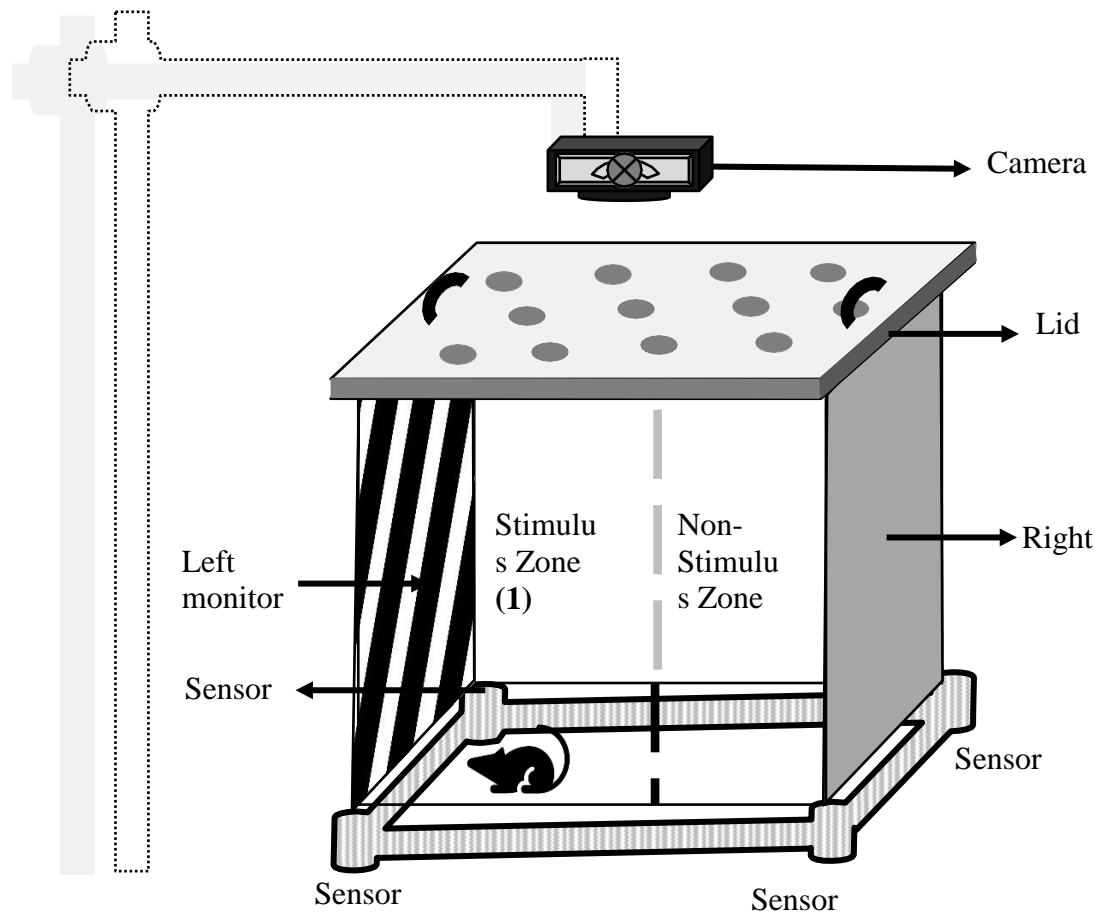
To test whether Δ Fos-B, a marker for neuronal activity, is higher in the visual cortex of J20 mice compared to control, we perfused a subset of our J20 (n = 6 mice) and WT (n = 6 mice) mice one day after the remote memory test and performed immunohistochemistry for Δ Fos-B (Figure 13 B and C). Preliminary analysis shows a 50% increase in Δ Fos-B immunofluorescence in the J20 mice compared to WT mice (Figure 13 D). These results indicate that J20 mice may have aberrant neuronal activity in the primary visual cortex.

3.6 Visual deprivation improves recent memory in WT but impairs it in J20 mice

To determine whether suppressing neuronal activity elicited by visual experience irrelevant to the visual memory task would improve memory in WT and J20 mice, we housed the mice in total darkness except during the VRM task. Since mice are nocturnal, we first tested whether absence of light would alter their home cage activity during dark adaptation over a one-week period. To this end, we monitored movement of four C57BL6J mice housed in a single cage using infrared sensors. As expected, we observed more movement of these mice between 6 PM – 6 AM (dark cycle) during the one week of the 12 h light/dark cycle (figure 14). We did not find any qualitative change to the home cage movement of the same mice when they are housed for the next one week in 24 h dark conditions (Figure 14).

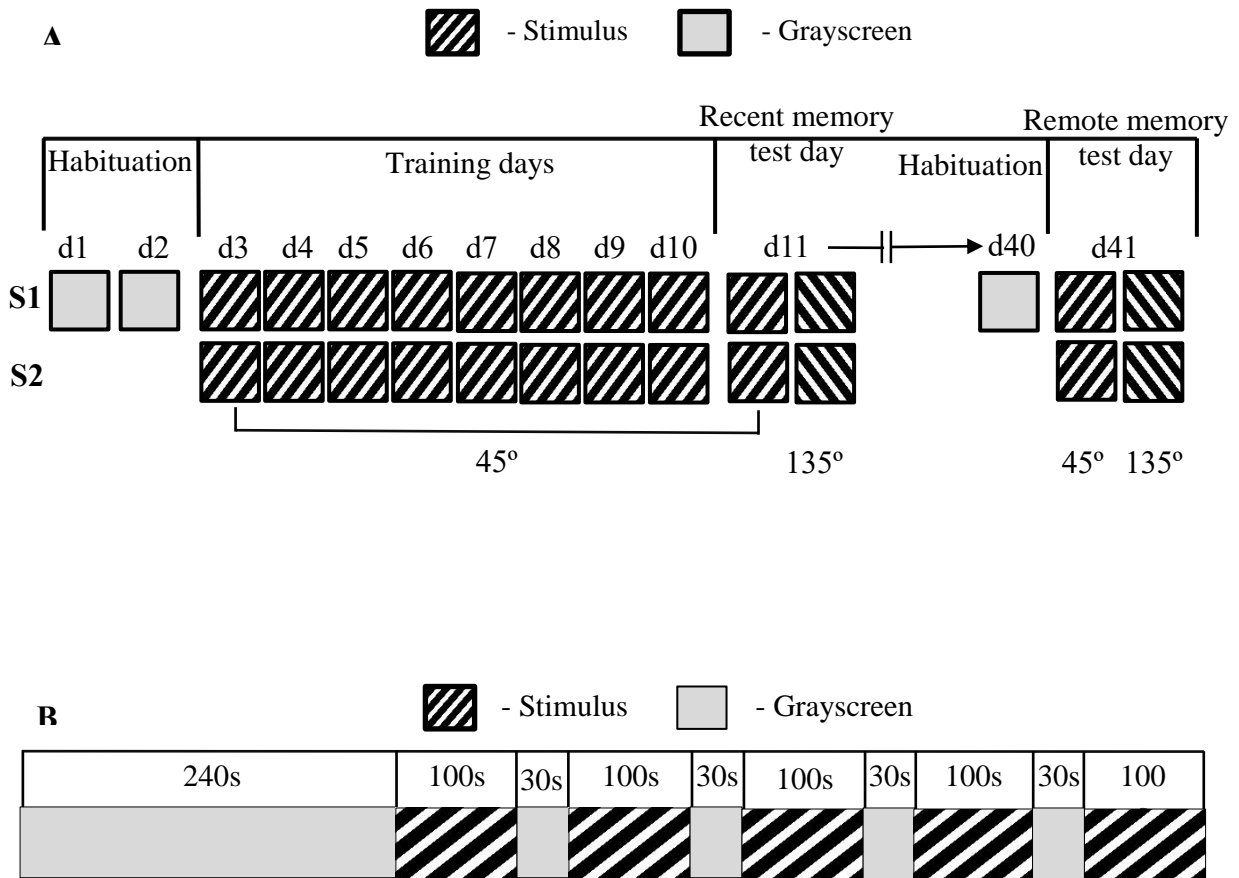
We next tested the effect of suppression of irrelevant visual experience on VRM in J20 and WT mice. Both WT (n = 6) and J20 mice (n = 3) exhibit stimulus (45 °) exploration on the first day suggesting that dark adaptation did not influence the exploration behavior of these mice. Interestingly, on the recent memory test day, J20 mice exhibited decreased preference for stimulus exploration whereas WT mice did not (Figure 15 A and B). Both mice responded to novel stimulus (135 °) by increased exploration of the stimulus (Figure 15.C). These preliminary results suggest that irrelevant visual experience promotes memory in WT mice but impairs it in J20 mice because in its absence J20 mice display normal memory whereas WT mice have impaired memory.

Figure 1. Description of arena and recording units



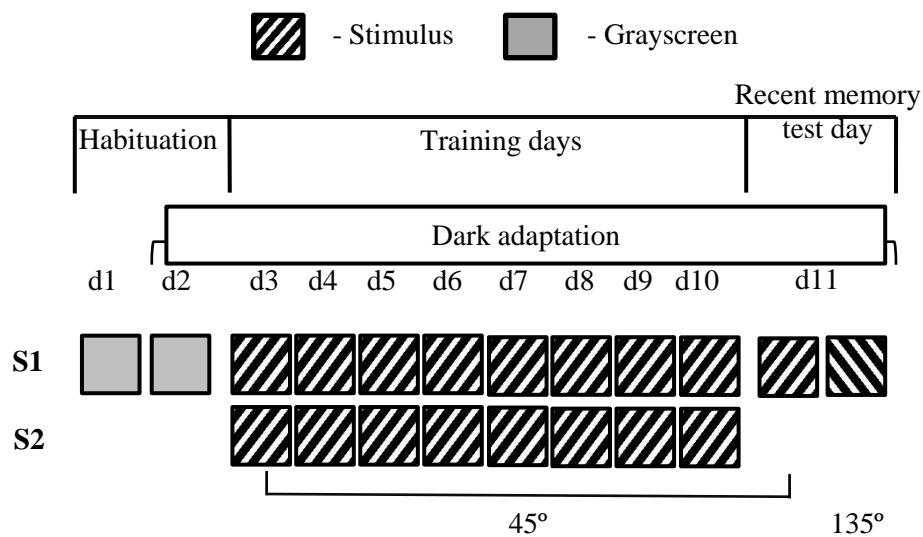
Opposite walls of the chamber are mounted with identical monitors (left monitor & right monitor) and enclosed with a lid with holes for ventilation. The arena is divided in two equal zones (stimulus zone **(1)** and non-stimulus zone **(2)**). Stimulus zone is the half closest to the monitor (randomly chosen for each session, stimulus is displayed on left monitor for representative purposes) displaying the grating stimulus. Four sensors (indicated as sensors 1 – 4) at the four corners of the 42-inch square sensing give results in the form of force displacement in mm from the center. The corresponding coordinates obtained are in the range of +210 to -210 mm for x & y co-ordinates each. A camera records the entire session and is mounted on a stand from the left side of the arena.

Figure 2: Visual Recognition memory paradigm.



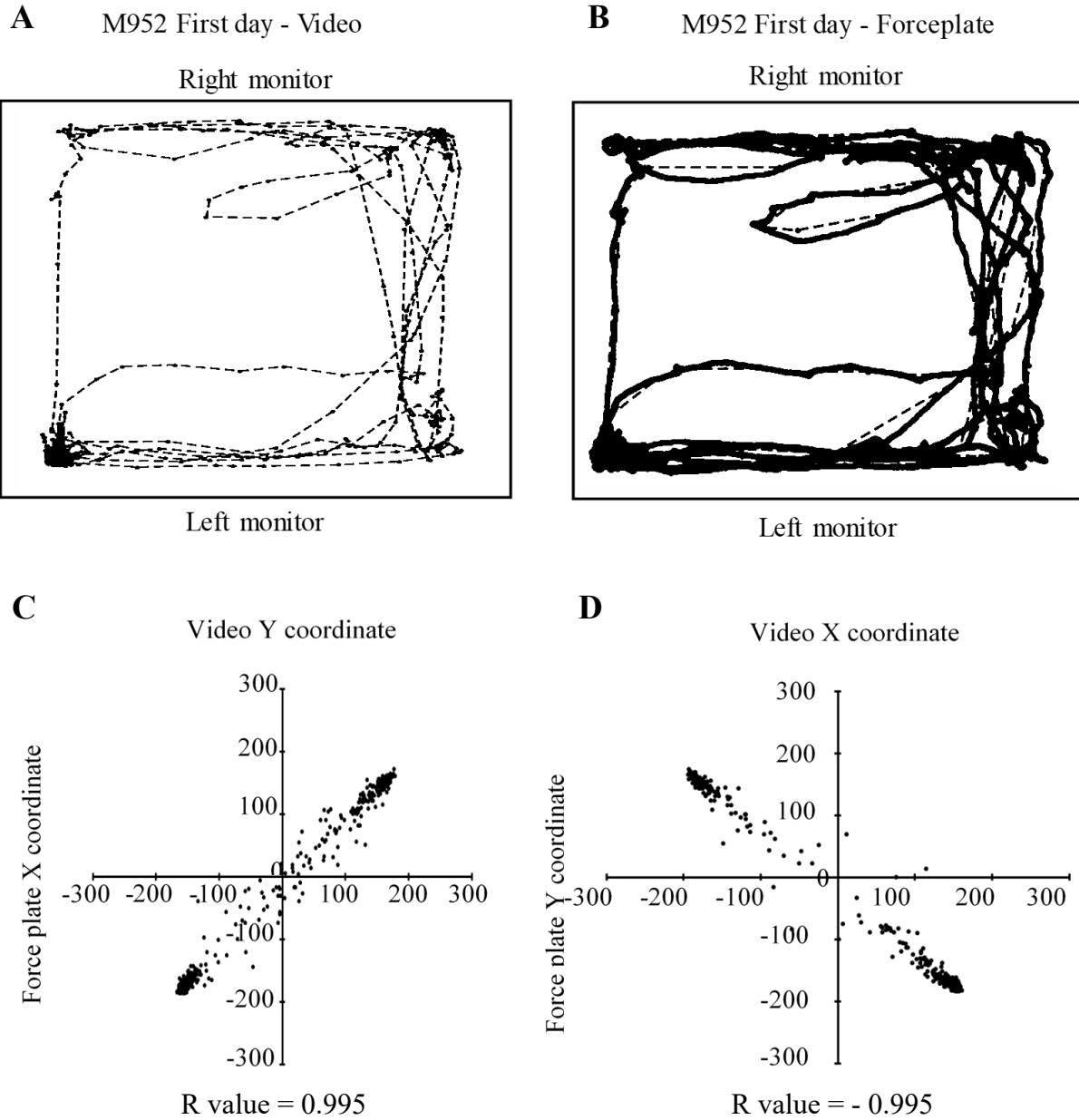
A. Timeline for the paradigm. Days 1-41 are indicated and S1 and S2 are session 1 and 2 respectively. **B.** Each stimulus session is divided into specific blocks: 4 minutes of grayscale (B) on both monitors, followed by five blocks of stimulus in one of the monitors. The other monitor remained gray. Phase reversing grating stimulus was presented for 100s/block, separated with 30-second inter-block gray screen intervals.

Figure 3: VRM paradigm – Dark adaptation



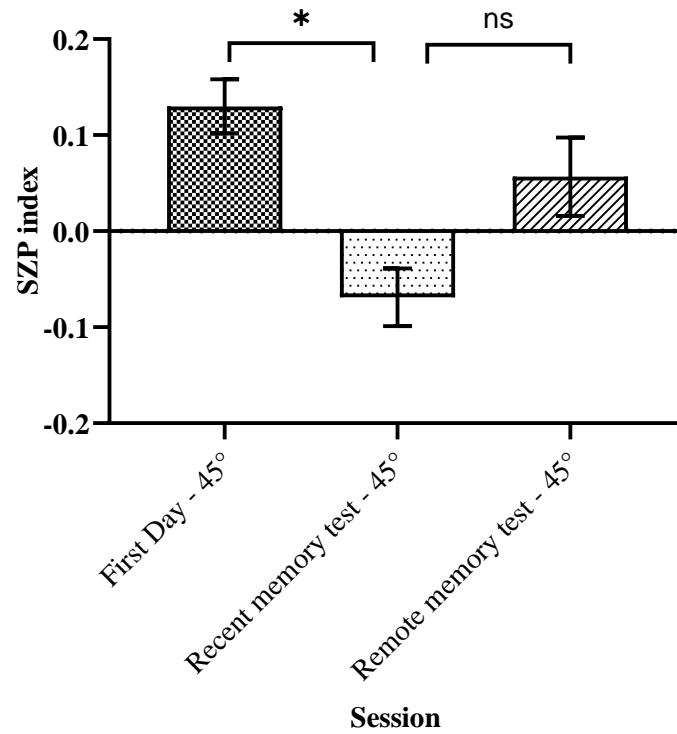
Timeline for the paradigm. Days 1-11 are indicated and S1 and S2 are session 1 and 2 respectively. After one day of habituation, except during VRM task, the mice are housed in dark conditions.

Figure 4: Validation of Forceplate Actometer



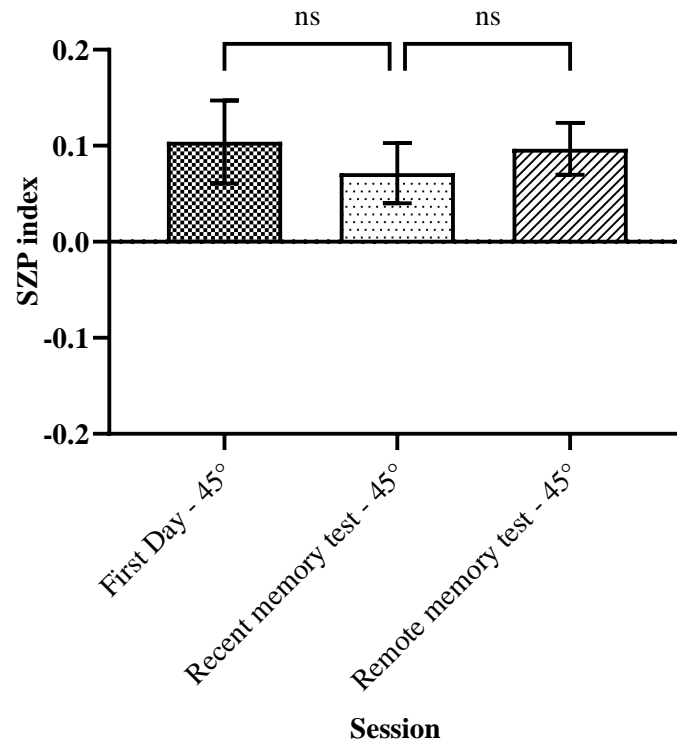
(**A** and **B**) representative traces for mouse # - M952 for first day of stimulus presentation obtained using a camera (**A**) and a Forceplate (**B**). (**C** and **D**) are scatter plots of correlation between X-Y coordinates obtained from the force plate and the camera for session The arena in the camera is shifted 90° to the left compared to the force plate (force plate negative X = video negative Y and force plate negative Y = video positive X). Also refer Table 3.1.1

Figure 5: Visual Recognition memory in Wild type male mice



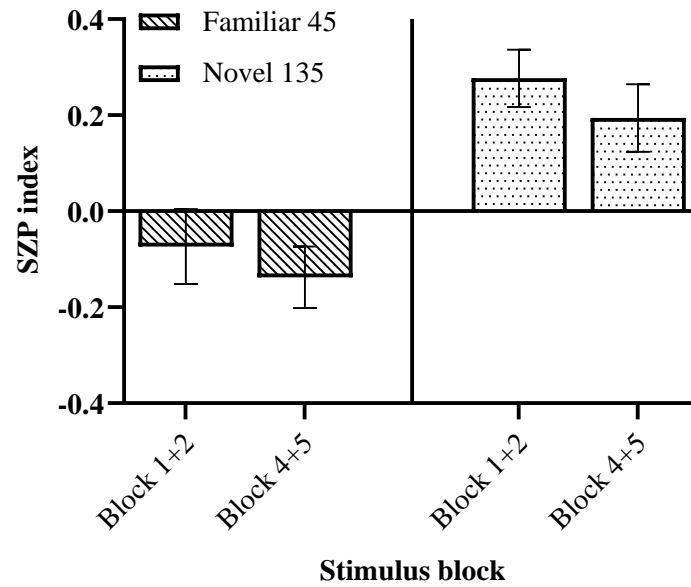
Visual Recognition Memory in Wild type mice (n=9). Session 1 data are presented as mean \pm SEM. Test day-stimulus angle is indicated in the X-axis SZP indicates stimulus zone preference. * $P < 0.05$, ns – non-significant by Repeated measures, One-way ANOVA with post-hoc Tukey's test. Also refer Table 4

Figure 6: Visual Recognition memory in J20 male mice



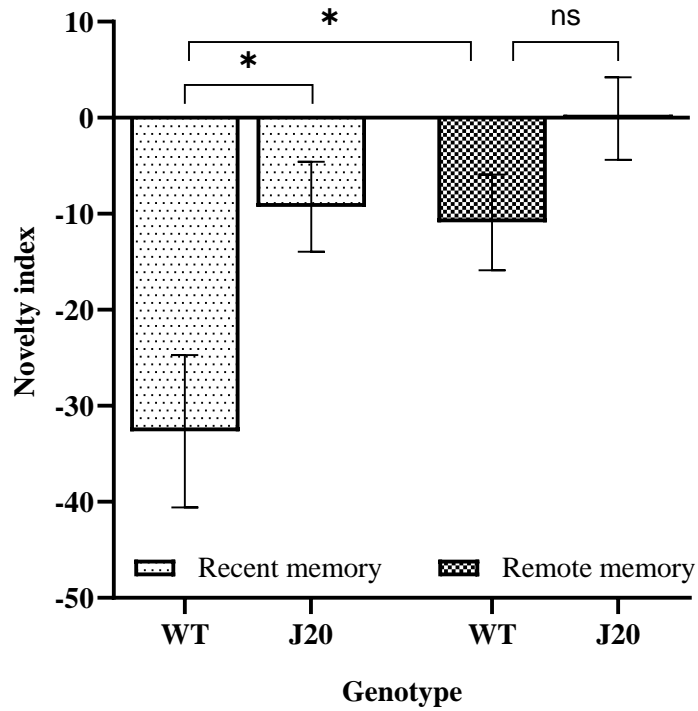
Visual Recognition Memory in J20 mice (n = 12). Session 1 data are presented as mean \pm SEM. Test day-stimulus angle is indicated in the X-axis. SZP indicates stimulus zone preference. ns – non-significant by Repeated measures, One-way ANOVA. Also refer table 5

Figure 7: Working memory doesn't influence stimulus zone preference



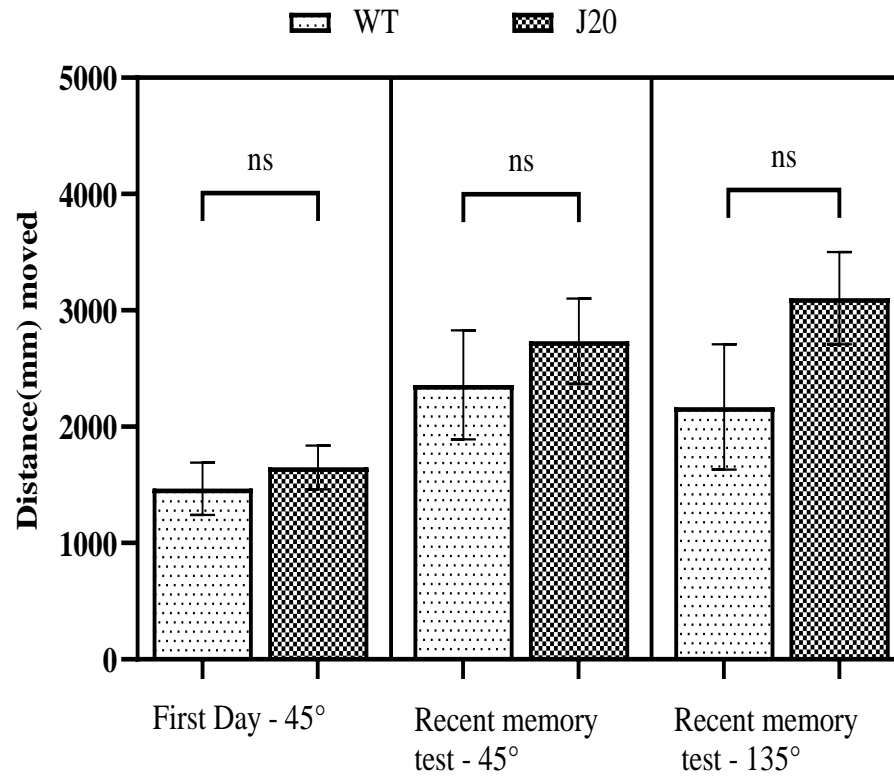
Block 1+2 and Block 4+5 represent the first two and the last two blocks of the five blocks stimulus in a session on recent memory test day for Wild type mice ($n = 9$). SZP refers to stimulus zone preference. Data are presented as mean \pm SEM.

Figure 8: Novelty index for grating stimulus on recent memory test day



Novelty index, calculated as the percentage decrease in time spent exploring familiar stimulus zone compared to novel stimulus zone on the same day for each mouse. Data are presented as mean \pm SEM for wild type (WT; n = 9) and J20 (n = 12) mice. Also refer Table 6

Figure 9 : Distance travelled in the arena during stimulus presentation by genotype



Data represents mean distance moved during stimulus presentation \pm SEM for each genotype (WT (n=9) and J20 (n=12)) on different days. Test day-stimulus angle is indicated in the X-axis. ns – non-significant by One-way Nested ANOVA . Also refer Table 7

Figure 10: Stimulus exploration occurs regardless of the side of stimulus presentation

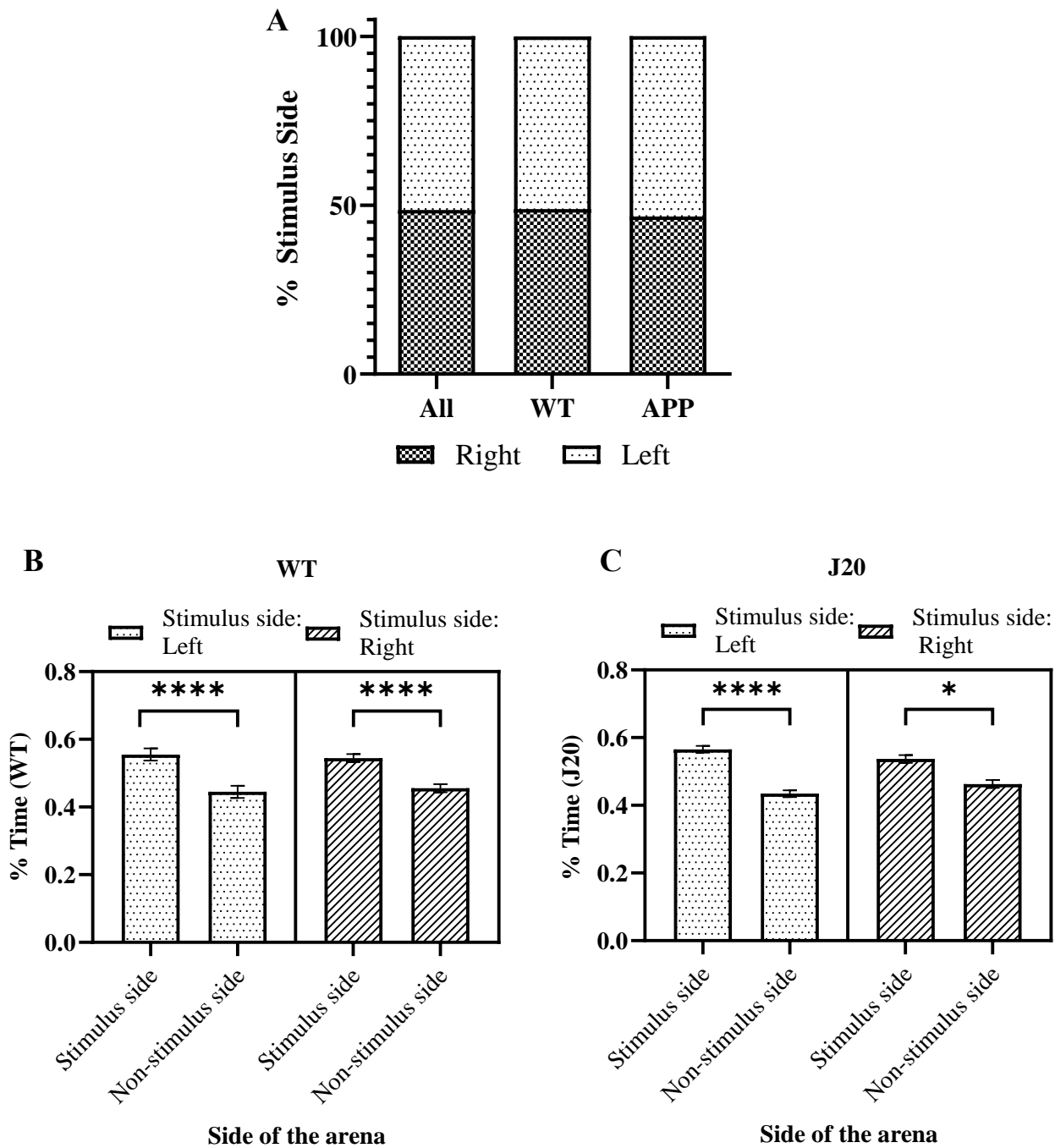
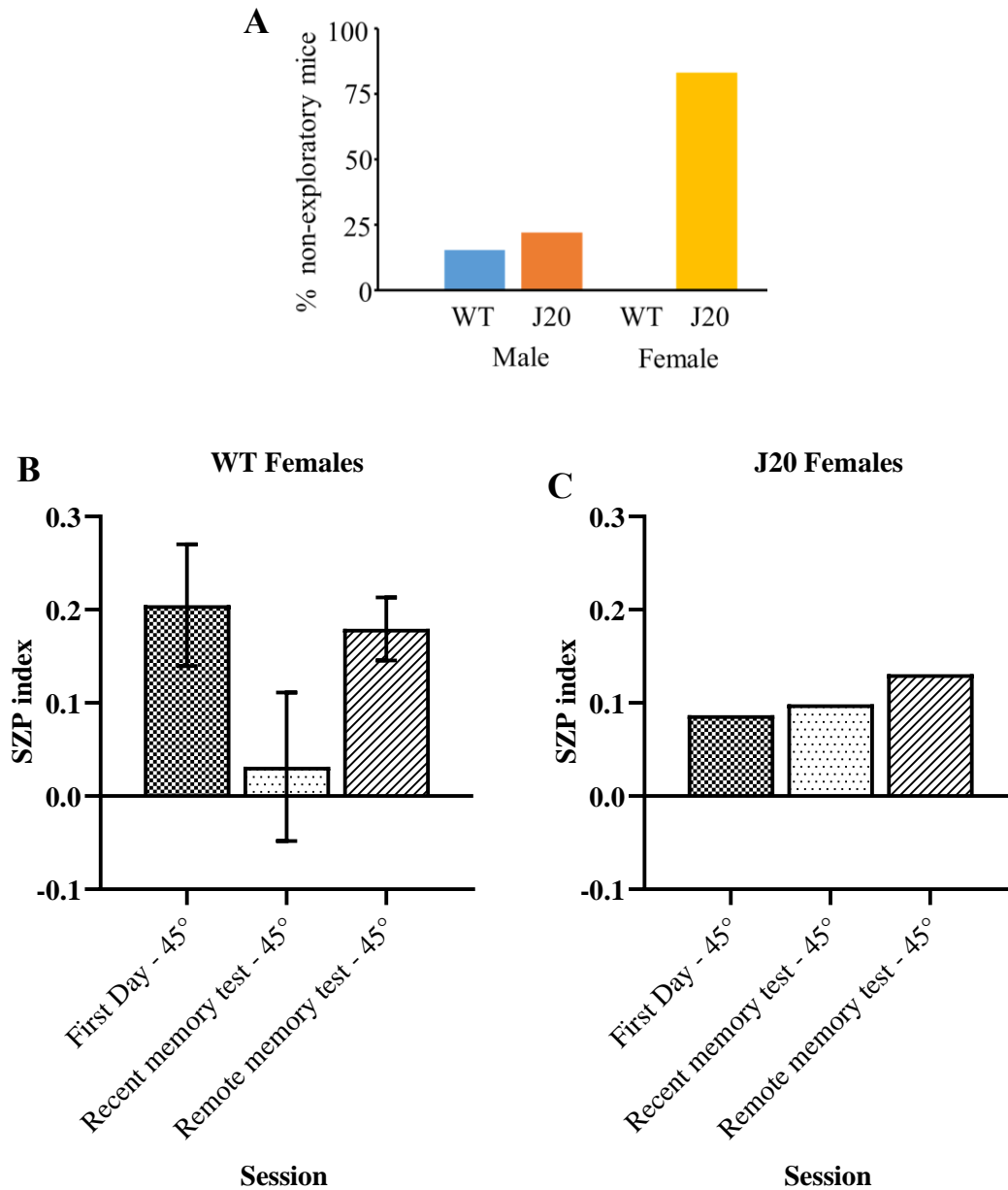


Figure 11: Visual memory paradigm in J20 female mice

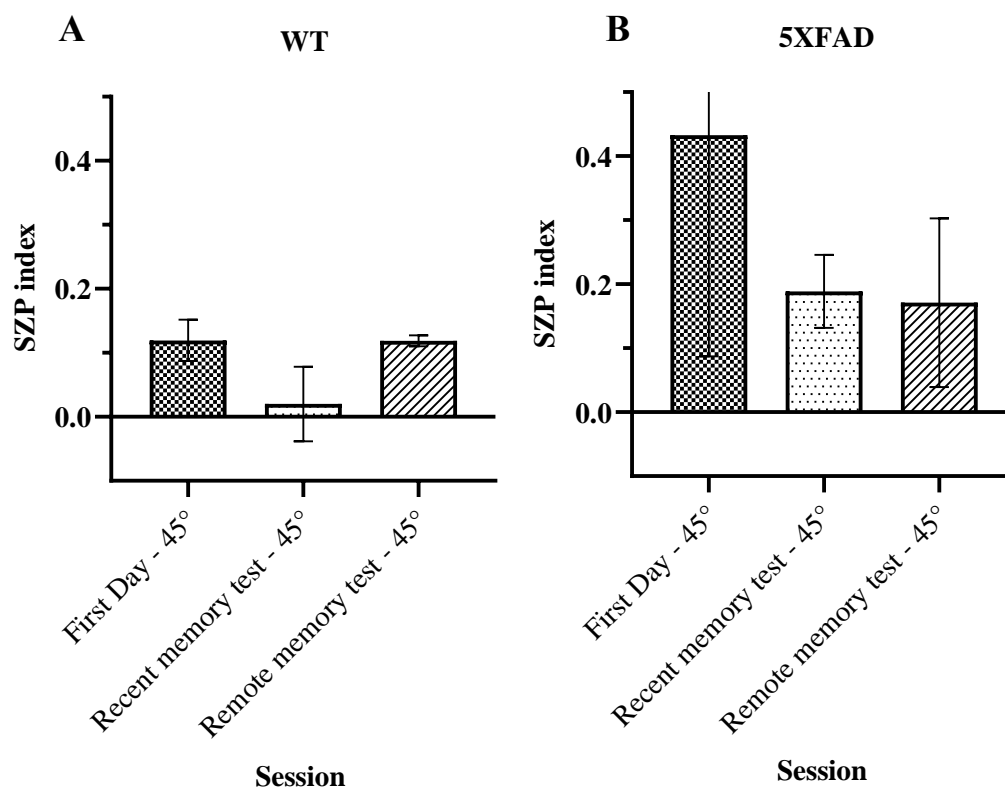


A. Percentage non-exploratory male (WT = 9, J20 = 12) and female (WT = 4, J20 = 6) mice.

Stimulus zone preference (SZP) index of wild type (WT, n=3; **B**) and J20 (n = 1; **C**) mice.

Test day-stimulus angle is indicated in the X-axis. Data are presented as mean \pm SEM.

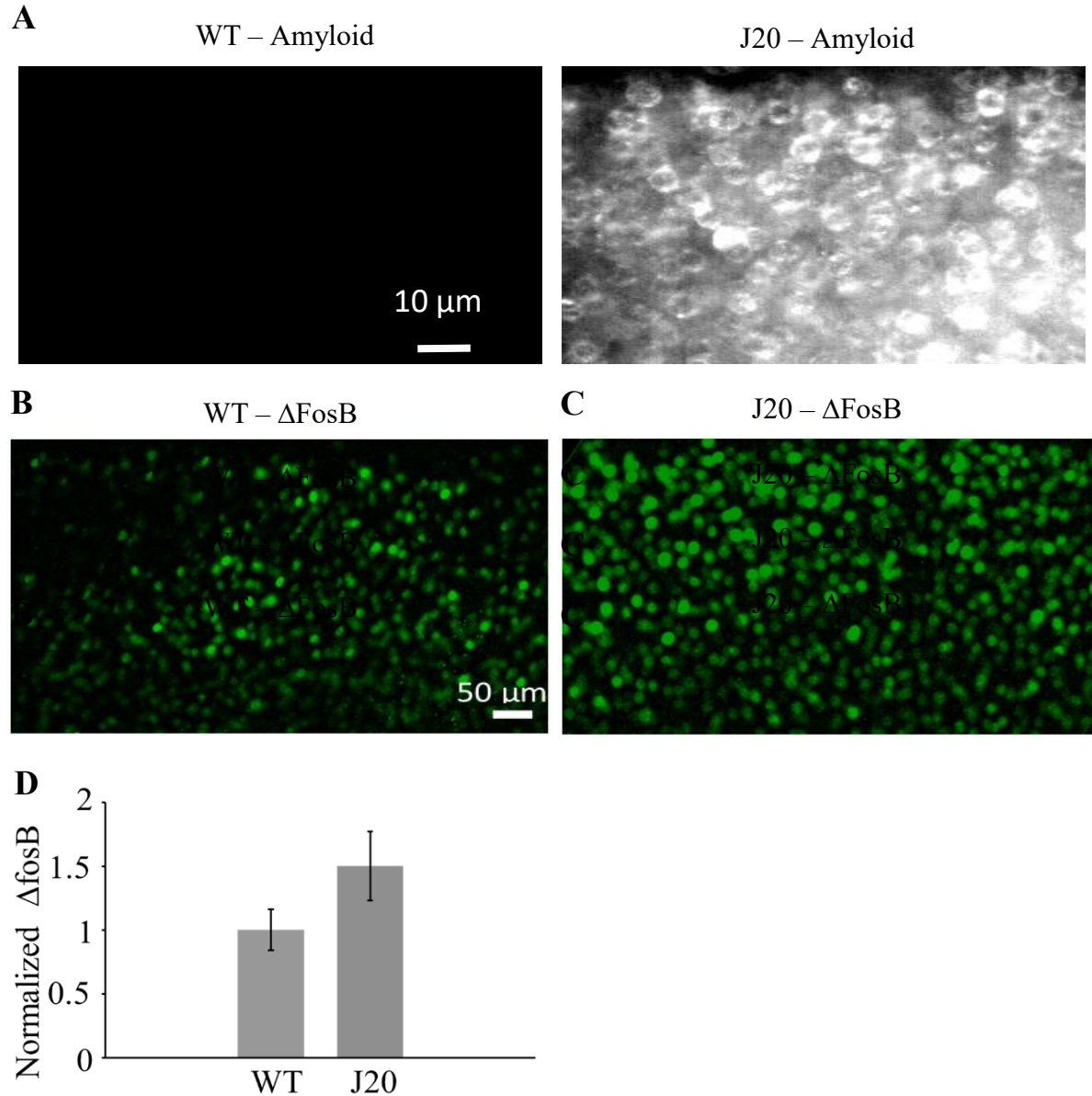
Figure 12: Visual memory paradigm in 5XFAD male



Stimulus zone preference (SZP) index of wild type (WT, n = 3; **A**) and 5X-FAD (n=2; **B**) mice.

Test day-stimulus angle is indicated in the X-axis. Data are presented as mean \pm SEM.

Figure 13: APP overexpression and neuronal hyperactivity in the visual cortex of J20 mice

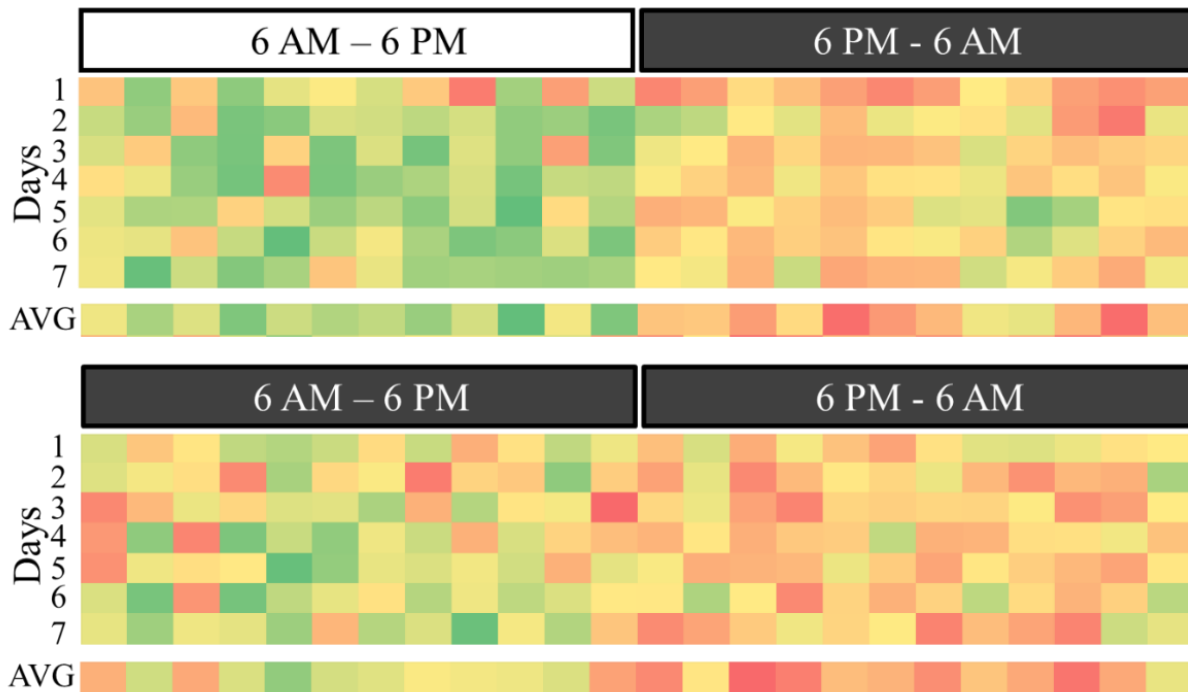


(A) Immunohistochemistry of amyloid in the visual cortex of wild type (WT) and J20 mice.

(B and C) Immunohistochemistry of Δ FosB in the visual cortex of WT **(B)** and J20 mice **(C)**

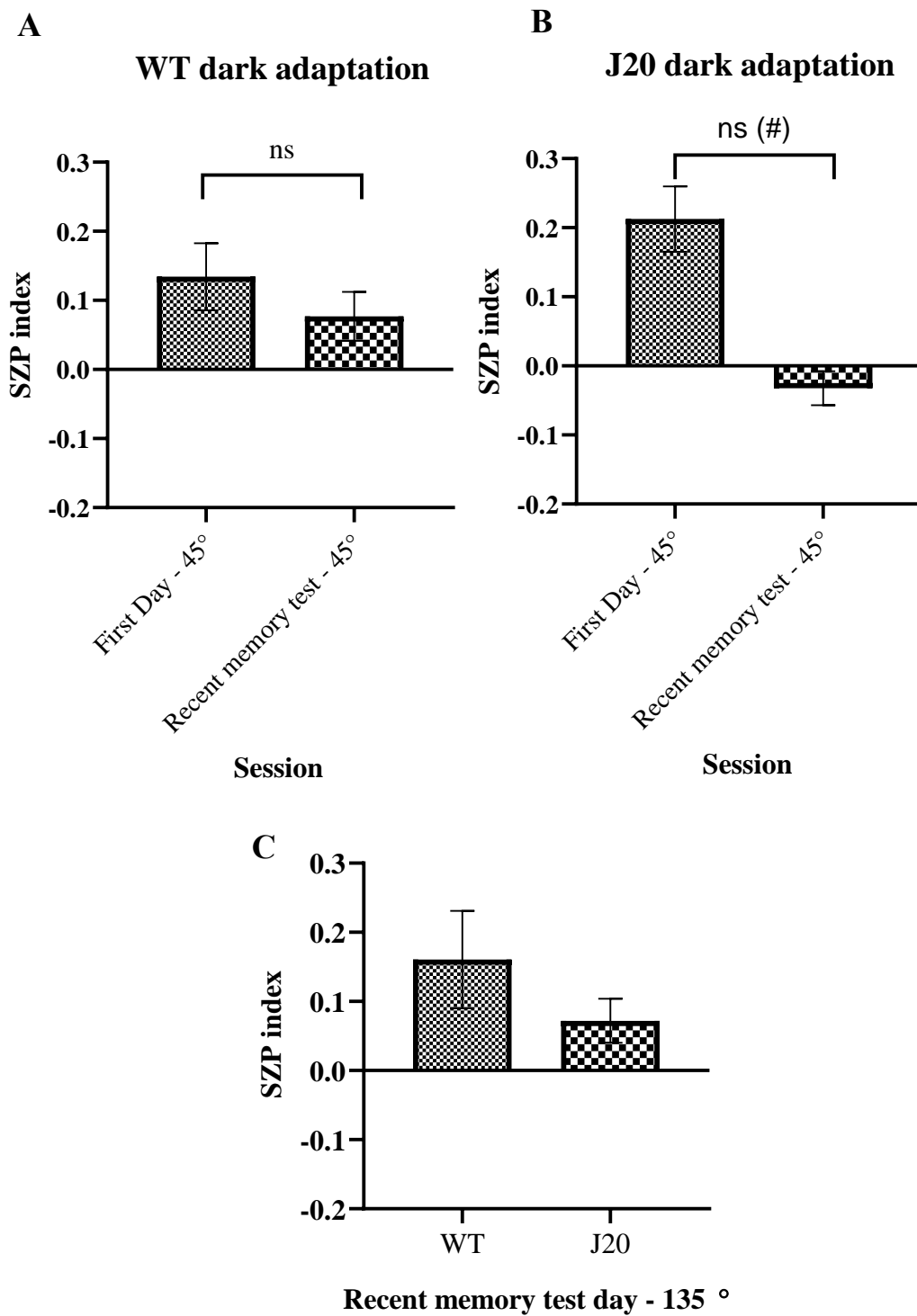
in the visual cortex. **D.** Quantification of Δ FosB immunofluorescence (n = 6 mice each for WT and J20). Δ FosB immunofluorescence for each mouse was normalized to the mean WT immunofluorescence (Normalized Δ FosB – y axis)

Figure 14: Home cage movement recorded before and during visual deprivation



Heat map of mouse movement in the home cage under 12 h light/dark cycle (top) and 24 h darkness (bottom). Each rectangle represents total movement during a one hour block. The frequency of mice movement is color coded from light green (lowest) to dark red (highest). AVG represents average of day1 – day 7 movement for each hour

Figure 15: The effect of irrelevant visual experience on the VRM



Preliminary data on the stimulus zone preference (SZP) index of wild type WT (n = 6; **A**); and J20 (n = 3; **B**) mice. Test day-stimulus angle is indicated in the X-axis. Data are presented as mean \pm SEM. ns – non significant ($P > 0.1$) and # ($P = 0.07$), by paired t-Test. Also refer 10. **C** is the SZP data for wild type WT (n = 6); and J20 (n = 3) mice on Recent memory test – 135°. Genotype is indicated in the X-axis. Data are presented as mean \pm SEM

Table 1. Correlation data for Figure 3

Sr. No.	Name of Session	R value for X coordinate of video vs Y coordinate of Forceplate	R value for Y coordinate of video vs X coordinate of Forceplate
1	M952D1S1	-0.995114118	0.99542677
2	M946IMF1	-0.975459704	0.97528208
3	M951D1S1	-0.985319579	0.98968682
4	M923LMN1	-0.966179419	0.973371436

Table 2. P values associated with Figure 4

Tukey's multiple comparisons test	Adjusted P Value	Statistical test
First Day - 45° vs. Recent memory test - 45°	0.0109	Repeated measure One-Way ANOVA
First Day - 45° vs. Remote memory test - 45°	0.0679	Repeated measure One-Way ANOVA
Recent memory test - 45° vs. Remote memory test - 45°	0.1311	Repeated measure One-Way ANOVA

Table 3. P values associated with Figure 5

Tukey's multiple comparisons test	Adjusted P Value	Statistical test
First Day - 45° vs. Recent memory test - 45°	0.7817	Repeated measure One-Way ANOVA
First Day - 45° vs. Remote memory test - 45°	0.988	Repeated measure One-Way ANOVA
Recent memory test - 45° vs. Remote memory test - 45°	0.8602	Repeated measure One-Way ANOVA

Table 4. P values associated with Figure 8

Tukey's multiple comparisons test	Adjusted P Value	Statistical test
Recent memory WT vs. Recent memory AD	0.015	Mixed model ANOVA
Recent memory WT vs. Remote memory WT	0.0428	Mixed model ANOVA
Recent memory WT vs. Remote memory AD	0.0006	Mixed model ANOVA
Recent memory AD vs. Remote memory WT	0.992	Mixed model ANOVA
Recent memory AD vs. Remote memory AD	0.5287	Mixed model ANOVA
Remote memory WT vs. Remote memory AD	0.4286	Mixed model ANOVA

Table 5. B.1 P values associated with Figure 10.B

Tukey's multiple comparisons test	Adjusted P Value	Statistical test
Right - Stimulus side vs. Left - Non-stimulus side	0.0182	Mixed model ANOVA
Right - Stimulus side vs. Left - Stimulus side	0.2984	Mixed model ANOVA
Right - Stimulus side vs. Right - Non-stimulus side	<0.0001	Mixed model ANOVA
Left - Non-stimulus side vs. Left - Stimulus side	<0.0001	Mixed model ANOVA
Left - Non-stimulus side vs. Right - Non-stimulus side	0.2984	Mixed model ANOVA
Left - Stimulus side vs. Right - Non-stimulus side	<0.0001	Mixed model ANOVA

Table 6. C.1 P values associated with Figure 10.C

Tukey's multiple comparisons test	Adjusted P Value	Statistical test
Right - Stimulus side vs. Left - Non-stimulus side	0.0081	Mixed model ANOVA
Right - Stimulus side vs. Left - Stimulus side	0.9702	Mixed model ANOVA
Right - Stimulus side vs. Right - Non-stimulus side	0.0001	Mixed model ANOVA
Left - Non-stimulus side vs. Left - Stimulus side	0.0001	Mixed model ANOVA
Left - Non-stimulus side vs. Right - Non-stimulus side	0.9702	Mixed model ANOVA
Left - Stimulus side vs. Right - Non stimulus side	0.0267	Mixed model ANOVA

Table 7. P values associated with Figure 15

Comparisons	Adjusted P Value	Statistical test
Recent memory test - 45° vs. First day - 45° (WT)	0.2658	Paired t-test
Recent memory test - 45° vs. First day - 45° (J20)	0.0762	Paired t-test

4. Discussion

Here we used a VRM paradigm to assess memory impairments under normal and amyloid pathology conditions. We found that WT mice form a VRM but do not retain it when tested one month after the training. J20 mice with amyloid pathology do not form this memory.

Interestingly, our preliminary data indicate that suppression of visual experience irrelevant to the memory reverses the phenotype – J20 mice form the VRM, whereas WT mice display impairments.

4.1 Natural forgetting in WT mice

An explanation for WT mice not retaining the VRM is that the memory decayed naturally over time due to cellular processes like protein turnover. Alternatively, other similar memories formed during the same period could have disrupted the original memory. There is strong evidence for memories, formed prior to (proactive) or after (retroactive) a memory task, in interfering with the stabilization of the newly formed memory [31]. Proactive and retroactive interferences are common methods used to induce forgetting under experimental settings. The VRM task does not include an active interference component and therefore, the loss of VRM one month following the task is indicative of natural decay of the memory. However, a caveat to this conclusion is that we used a 135° stimulus to assess motivation on the test day following the familiar 45° stimulus presentation. It is possible that the presentation of 135° stimulus interfered with long-term consolidation of the memory of the 45° stimulus. Though it is a possibility, it is unlikely because the increase in VEP associated with repeated exposure to the 45° stimulus is not reduced even when it is followed by several days of 135° stimulus [94].

What makes some memories to decay over time? One possibility is that these memories are weakly consolidated and therefore, are lost easily over time. The factors that influence the consolidation of memories are not well understood. Memory acquisition and consolidation transiently increase neuronal excitability [98, 99]. This happens in the context of additional excitability induced by similar experiences irrelevant to the memory task. Since neurons maintain their activity levels within a dynamic range by regulating their excitability[100, 101], memory consolidation may be weakened to prevent epileptogenesis. For instance, consolidation of a spatial memory following a spatial memory task happens when the animal is having space perception irrelevant to the spatial memory task. As a consequence, spatial memory consolidation would add to neuronal excitability along with the memory irrelevant space perception. However, this is hard to test because neuronal activity associated with space perception is unavoidable following or prior to a spatial memory task.

The VRM task we used is uniquely suited to test our hypothesis because the VRM is associated with synaptic plasticity in the primary visual cortex [92], which is mostly unimodal and responsive to visual experience. Deprivation of visual experience at all times other than during the VRM task would prevent excitability induced by experience irrelevant to the VRM. Therefore, we expected VRM to be consolidated effectively for long term maintenance.

Surprisingly, we found that dark adaptation during the training period resulted in impaired memory even when tested one day after the training period. This suggests that excitability induced by irrelevant experience during training does not interfere with the memory, instead they promote memory acquisition during the training. However, it is still possible that irrelevant experience after training interferes with memory maintenance and this can be tested by dark adaptation after the training.

4.2 Impaired memory in J20 mice

What could be the mechanism of VRM deficit in J20 mice? Diffuse amyloid plaques in the neocortex of J20 mice arise by ~ 5 – 7 months of age[102]. Since we used 3 – 4-month old mice, the VRM impairment precedes the development of plaques. Our immunostaining reveals APP is overexpressed in the visual cortex of ~3-4-month old mice. APP overexpression has been shown to induce neuronal hyperactivity [103], and suppression of APP expression restores neuronal activity levels and excitatory and inhibitory (E/I) balance [69, 104]. Neuronal hyperactivity is also seen in AD and has been associated with memory deficits [71]. Interestingly, neuronal activity increases secretion of the amyloid beta peptide; a proteolytic fragment derived from APP and suppression of neuronal activity reduces its secretion [105]. Therefore, accumulation of extracellular amyloid peptide due to hyperactivity could impact synapses and memories.

Consistent with this idea, both the suppression of hyperactivity with the antiepileptic drug levetiracetam and the clearance of extracellular amyloid have been shown to improve memory [72]. Therefore, the VRM deficit in APP mice could stem from hyperactivity in the visual cortex of these mice, presumably caused by mutated APP overexpression. This view, however, is challenged by the findings that suppression of neuronal activity by diazepam, a GABA receptor agonist, impairs memory despite reducing extracellular amyloid levels [106]. Levetiracetam suppresses hyperactivity without disrupting normal neuronal activity whereas diazepam would suppress global neuronal activity and therefore, interfere with memory through other mechanisms [107].

APP induced hyperactivity could interfere with memory through mechanisms that do not involve amyloid secretion. Recently, elevated expression of Δ FosB, a protein that accumulates in

response to neuronal activity, was reported in J20 mice and was found to correlate with the cognitive deficit in these mice [76]. Consistent with such a mechanism, we also found elevated expression of Δ FosB in the visual cortex of J20 mice compared to WT control siblings. Since we performed Δ FosB immunohistochemistry at the end of the VRM task, it is unclear whether the reduction in immunofluorescence in WT mice reflects basal differences in Δ FosB expression between the genotypes or it is caused by the training experience. Δ FosB immunohistochemistry in mice that did not undergo the VRM task would address these questions.

APP mediated hyperactivity could occur spontaneously or be elicited by experience. Spontaneous hyperactivity is observed both in EEG recordings and during neuronal calcium imaging in mice with APP overexpression (APP mice) [69]. On the other hand, neuronal responses to experience are also elevated by sensory stimulation in APP mice [108]. The relative roles of spontaneous and experience-evoked hyperactivity in impairing memories are not known. Our preliminary data show that suppressing neuronal excitability by dark adaptation enable VRM in APP mice. These results suggest that experience-evoked activity could contribute to APP evoked hyperactivity and memory impairments. Experience deprivation by whisker ablation reduces amyloid plaques but interestingly, does not rescue synapse loss in APP mice [108]. The authors found that suppressing neuronal activity, while reducing extracellular amyloid, causes an increase in the levels of intracellular amyloid [106]. Increased intracellular amyloid levels are also associated with synapse loss [109]. If experience deprivation increases intracellular amyloid levels and decreases synapse numbers, how could dark adaptation improve VRM? In our paradigm, mice were dark adapted at all times other than during the VRM task. Therefore, unlike complete experience deprivation, the mice had visual stimulation during the training period.

These brief periods of visual stimulation may have been enough to prevent the intracellular buildup

of amyloid due to sensory deprivation [110, 111]. Visual deprivation could also have improved VRM through increased clearance of extracellular amyloid. Induction of gamma oscillations in the brain reduces amyloid levels and improves cognition [112]. APP mice display reduced gamma oscillation due to disruption of parvalbumin neuron excitability [113]. Visual deprivation was recently found to increase the power of gamma oscillation in the visual cortex [114]. Therefore, dark adaptation could have improved the power of gamma oscillations in J20 mice and restored the memory.

4.3 A model for the effect of irrelevant visual experience on visual memory

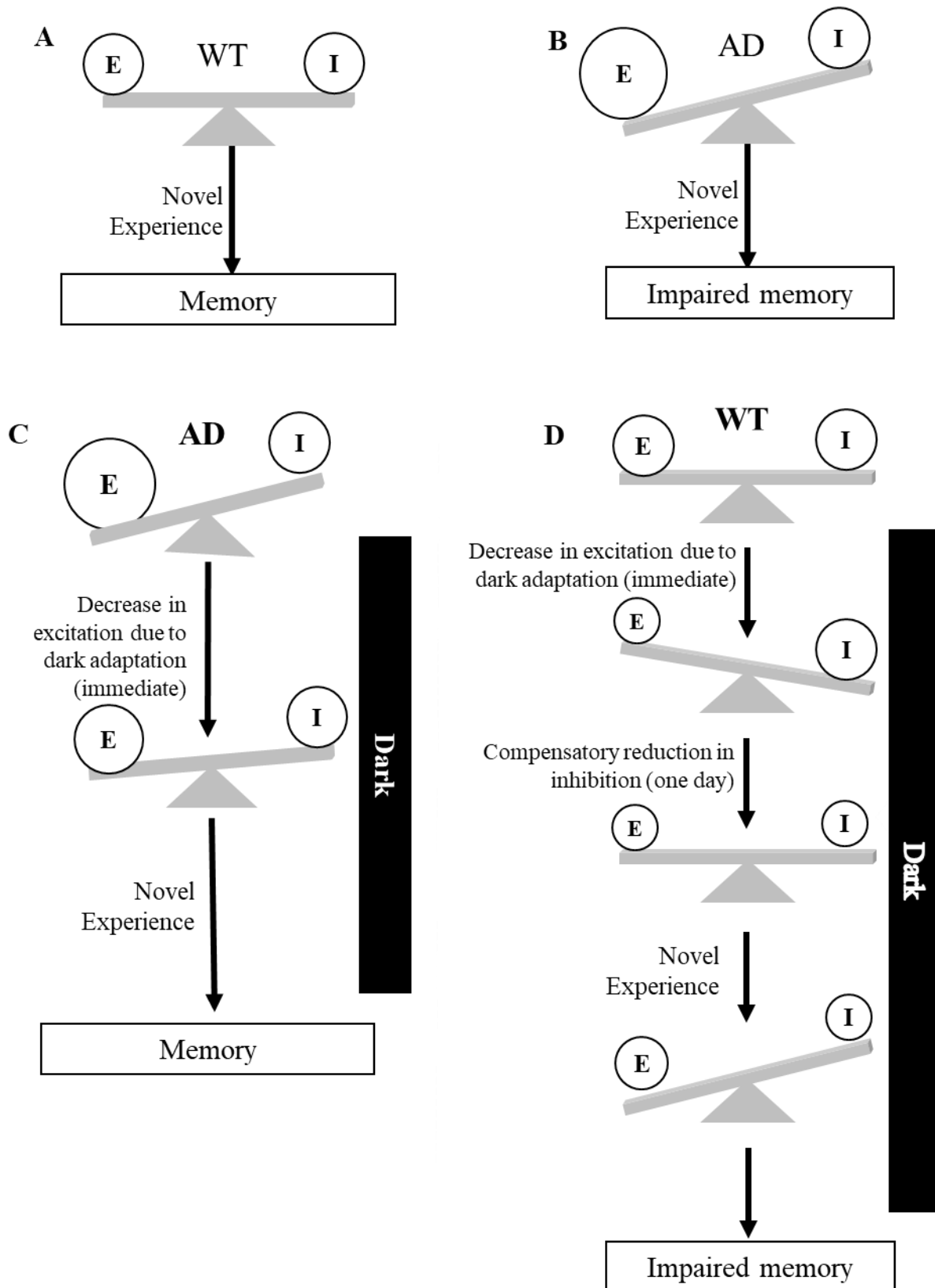
Irrelevant experience in the context of our study is defined as any related visual stimuli that activates partially or completely a component of the visual cortical neurons involved in the VRM task but is not relevant for VRM. Primary visual cortex neurons are involved in understanding orientations in our environment and hence show synaptic plasticity directly to the orientation stimulus in the VRM task. Thus naturally occurring orientations acts as related but irrelevant stimuli to the VRM task. One of the most surprising findings in our study is that reducing activity evoked by memory irrelevant visual experience resulted in disruption of VRM in the WT mice. This is in contrast to what was observed in J20 mice where suppression of task irrelevant experience improved VRM. How does suppression of task irrelevant experience flip memory phenotype of WT and J20 mice?

Neurons maintain their activity within a dynamic range by maintaining appropriate E/I balance [115]. Memory acquisition results in the strengthening of excitatory connectivity between neurons co-activated by an experience [116]. This is facilitated by the transient disinhibition of

these neurons [117]. Together, disinhibition and increased excitatory connectivity would push E/I balance towards more excitation. To maintain the memory and to maintain E/I balance, neurons would have to lose other excitatory synapses and undergo re- inhibition. Consistent with this idea, learning first increases excitatory synapse formation, and this is followed by a period of loss of other pre-existing synapses [25]. Thus, memory acquisition requires well-coordinated changes to E/I balance. J20 mice exhibit hyperactivity, presumably due to impaired E/I balance favoring excitation [69]. Memory acquisition in J20 mice would further push the E/I imbalance towards higher excitation and increase epileptiform activity. A failure to acquire or maintain memories, therefore would be an adaptation to maintain homeostasis and prevent epilepsy. Visual deprivation in J20 mice would reduce the experience-evoked excitability and restore E/I balance (Figure 16). This state is similar to WT mice, and therefore, J20 mice are able to form visual memory in dark. In contrast, reduced excitability due to visual deprivation in WT mice would impair E/I balance favoring more inhibition (Figure 16). However, our previous work found that under such conditions, inhibitory synapses are lost at a higher rate than normal (unpublished data). This is consistent with reduced inhibition observed in visual deprivation in other studies [118]. The reduced inhibition would compensate for reduced visually driven excitation and restore the E/I balance. Since inhibition is reduced in dark, a sudden increase in excitation during the VRM task would imbalance E/I towards more excitation, resulting in hyperactivity (Figure 16). Thus, WT mice undergoing VRM task during dark adaptation would be similar to J20 mice under normal light conditions. As a consequence, memory is impaired in WT mice. These data reveal an unexpected role for irrelevant visual experience in promoting the acquisition of visual memories, presumably by maintaining inhibition. An alternative possibility is that lack of visually driven activity downregulates activity dependent genes important for synaptic plasticity [119, 120].

Therefore, the machinery needed for synaptic plasticity may not be present at optimal levels to capture incoming information more effectively, and consequently, memories may be lost.

Figure 16: A model for the role of irrelevant visual experience on visual memory



A. WT mice with balanced excitation (E) and inhibition (I) exhibit normal memory acquisition. **B.** J20 mice exhibit neuronal hyperactivity, suggestive of impaired E/I balance. **C and D** Visual deprivation reduces the 'E' both in WT (**C**) and J20 (**D**) mice but evokes a compensatory 'I' reduction only in WT mice. Visual experience under conditions of reduced 'I' causes hyperactivity and disrupts memory in WT mice. Size of the circle reflects the magnitude.

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